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On combining microRNA analysis with DNA profiling in a single stream process

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Supervisor: Dr Graham Williams





FSF Emerging Forensic Scientist Award Oral Presentation

What are microRNAs and why are we interested in them?

Small (~22nt) non-coding RNAs

Regulate mRNA expression

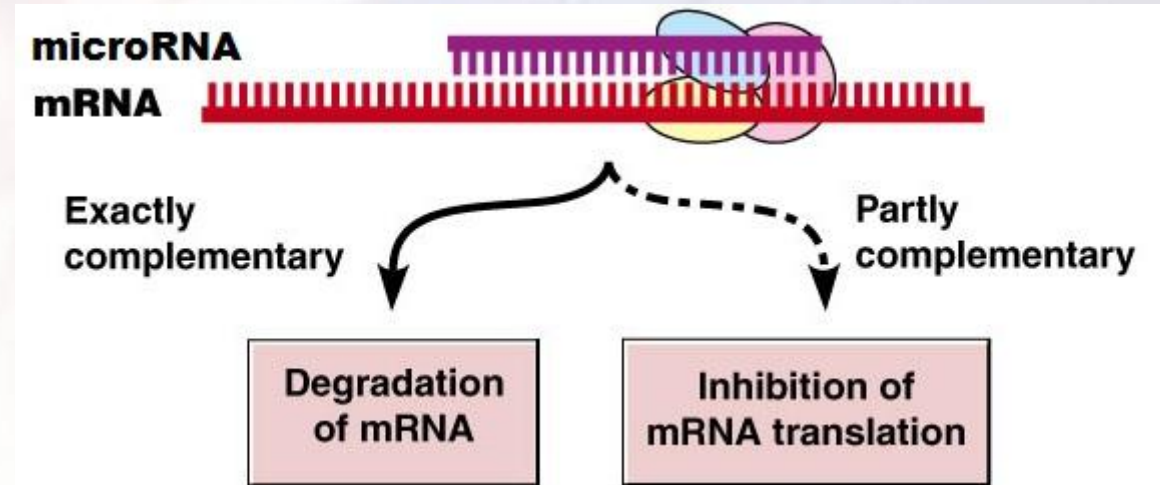
Advantages for forensic science

Stable

High expression levels

Sensitive and specific detection

Co-extracted with DNA



MicroRNAs can be used for body fluid identification

More than 2500 microRNAs in humans
Tissue specific expression patterns

Previously identified markers

Blood: miR-16a, miR-142 and miR-451a

Saliva: miR-203a and miR-205

Semen: miR-10a and miR-135a

Vaginal material: miR-1260b

Control: SNORD44

Improve current methodology with our novel method

Current

- microRNAs: RT-qPCR
 - Separate reaction per microRNA

Our novel method

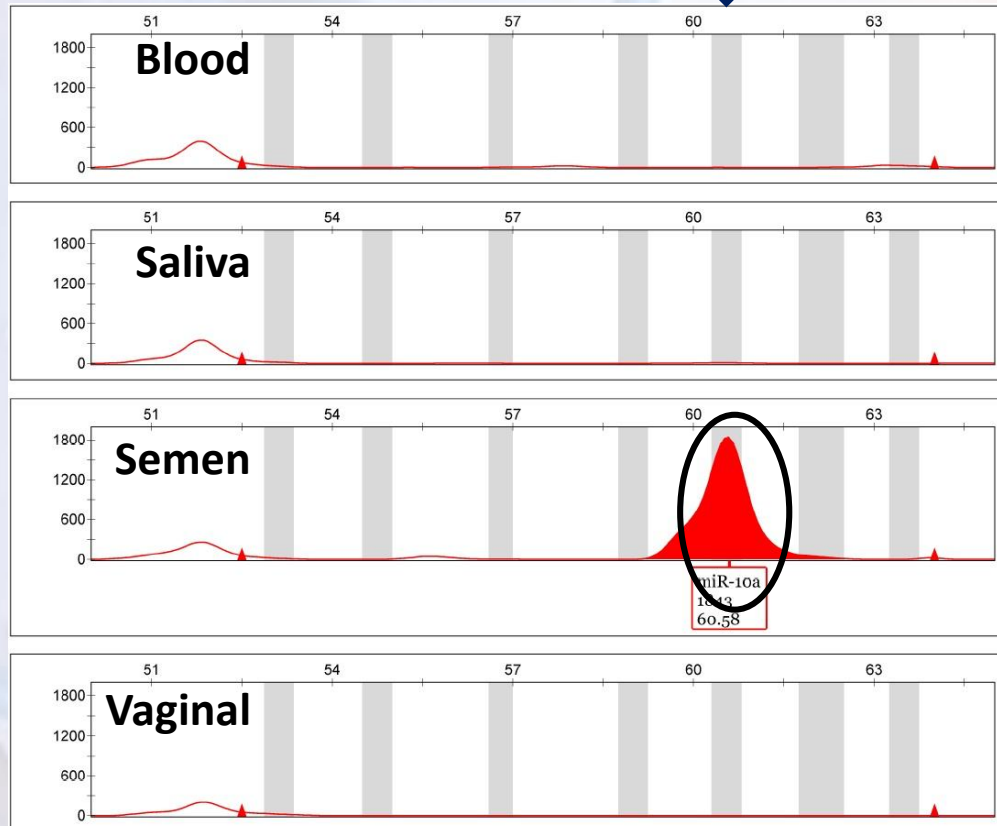
- Analyse microRNAs with capillary electrophoresis (CE)
 - Multiplex microRNAs in single test
 - Possibility to combine microRNA analysis with DNA profiling

Methods and materials

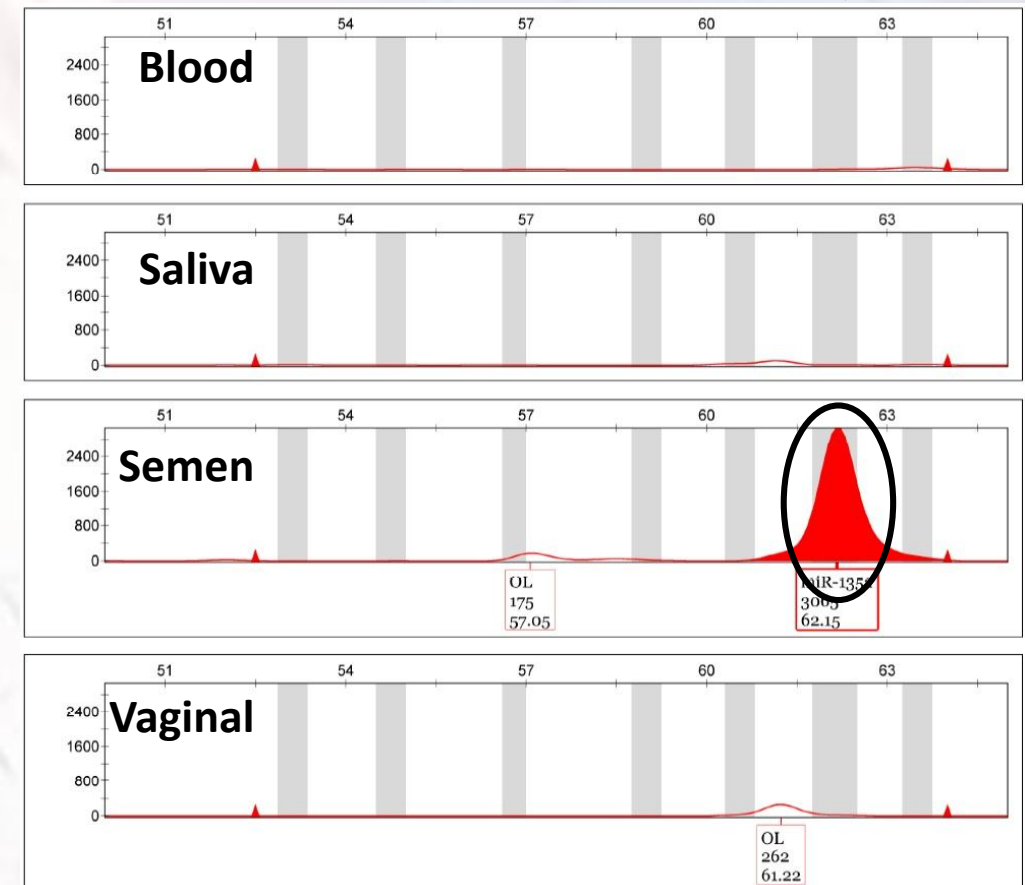
- 5 samples of 4 tissue types
 - Blood, saliva, semen and vaginal material
- DNA extraction
- Normalised to 0.5ng/ μ l human DNA
- Tested for 9 markers
- Multiplex stem-loop reverse transcription
- ROX-labelled primers

miR-10a and miR-135a are exclusively detected in semen

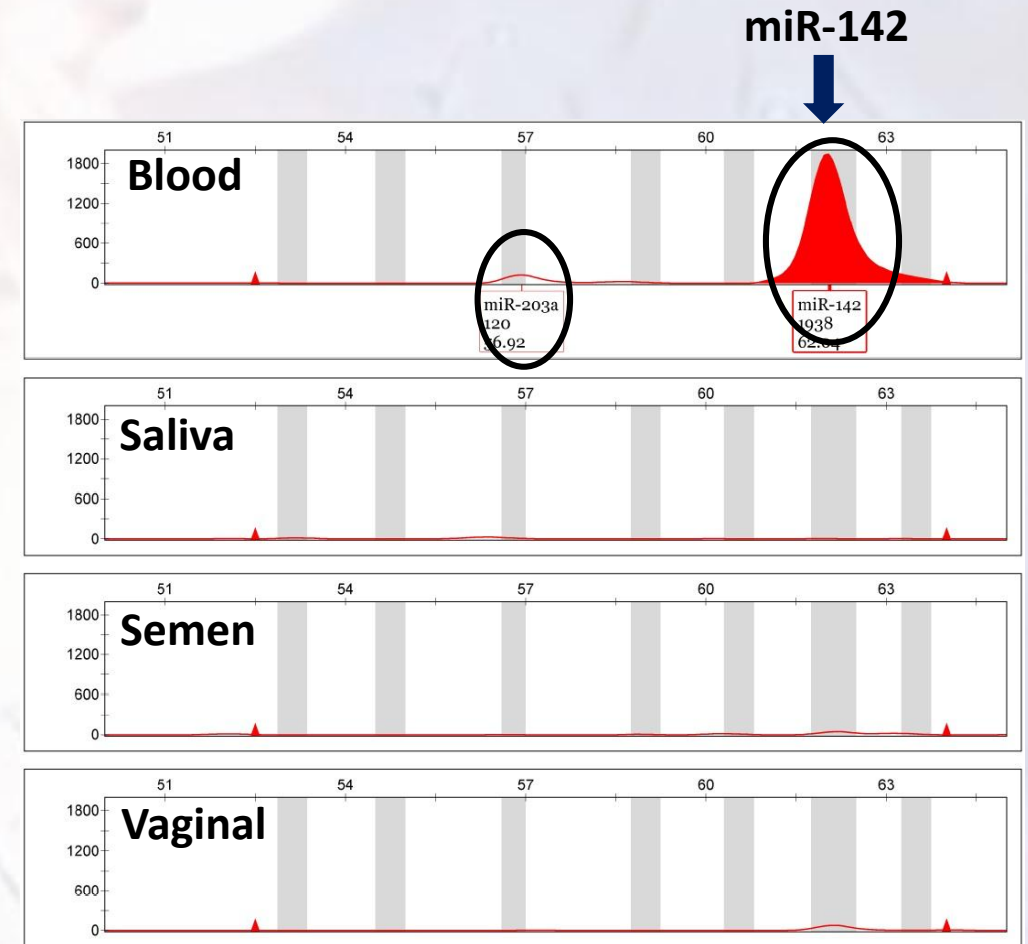
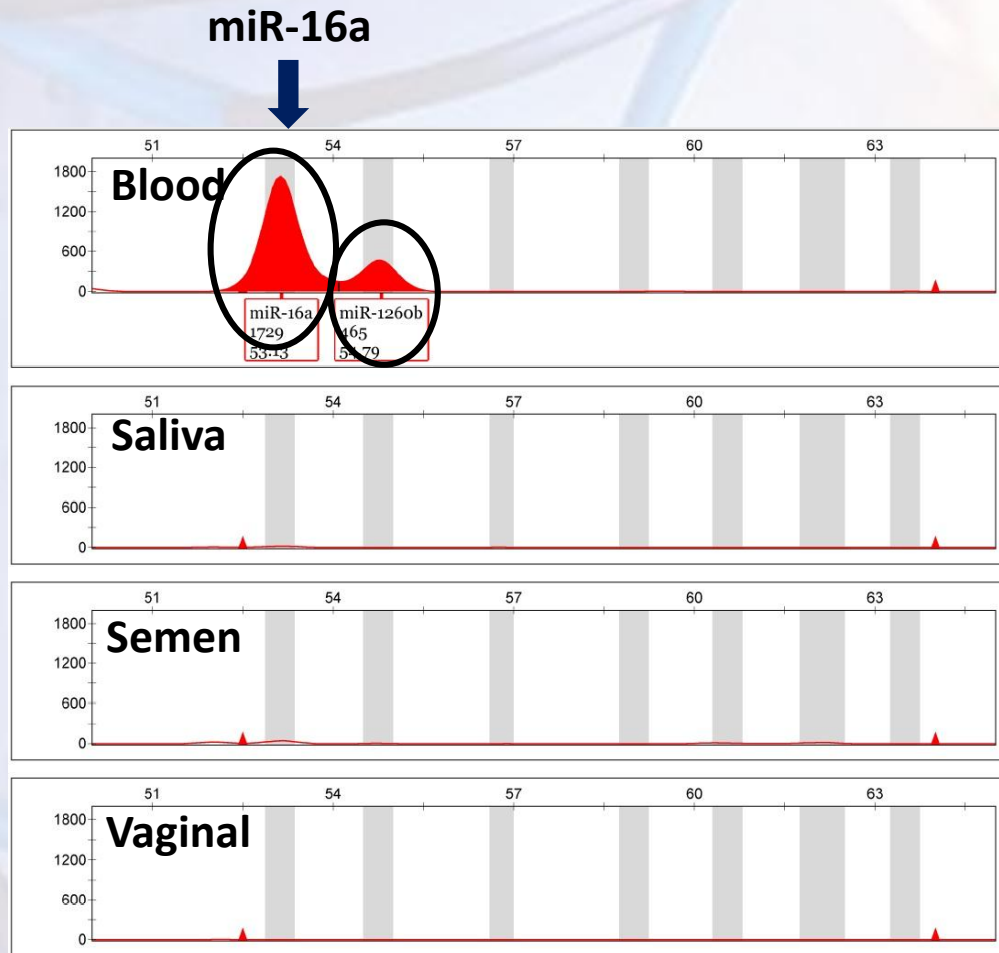
miR-10a



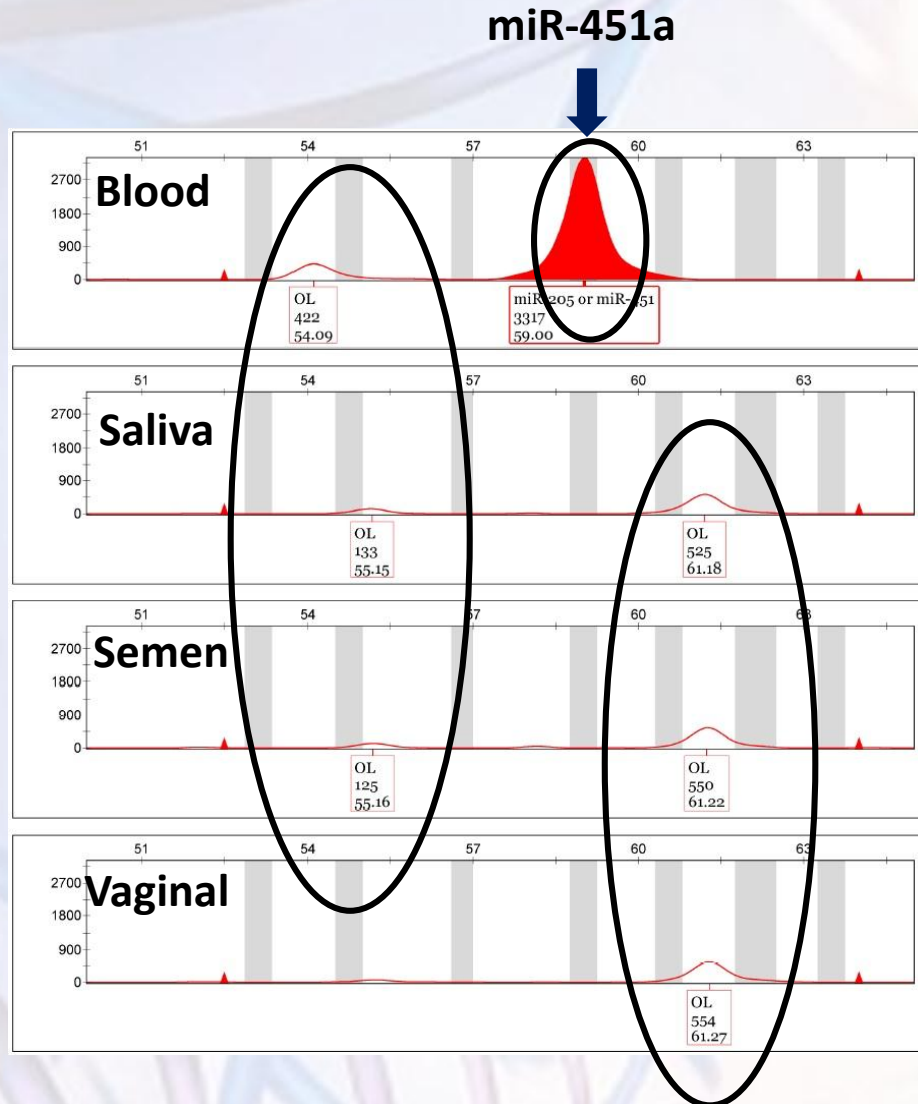
miR-135a



miR-16a and miR-142 are exclusively detected in blood



miR-451a is exclusively detected in blood



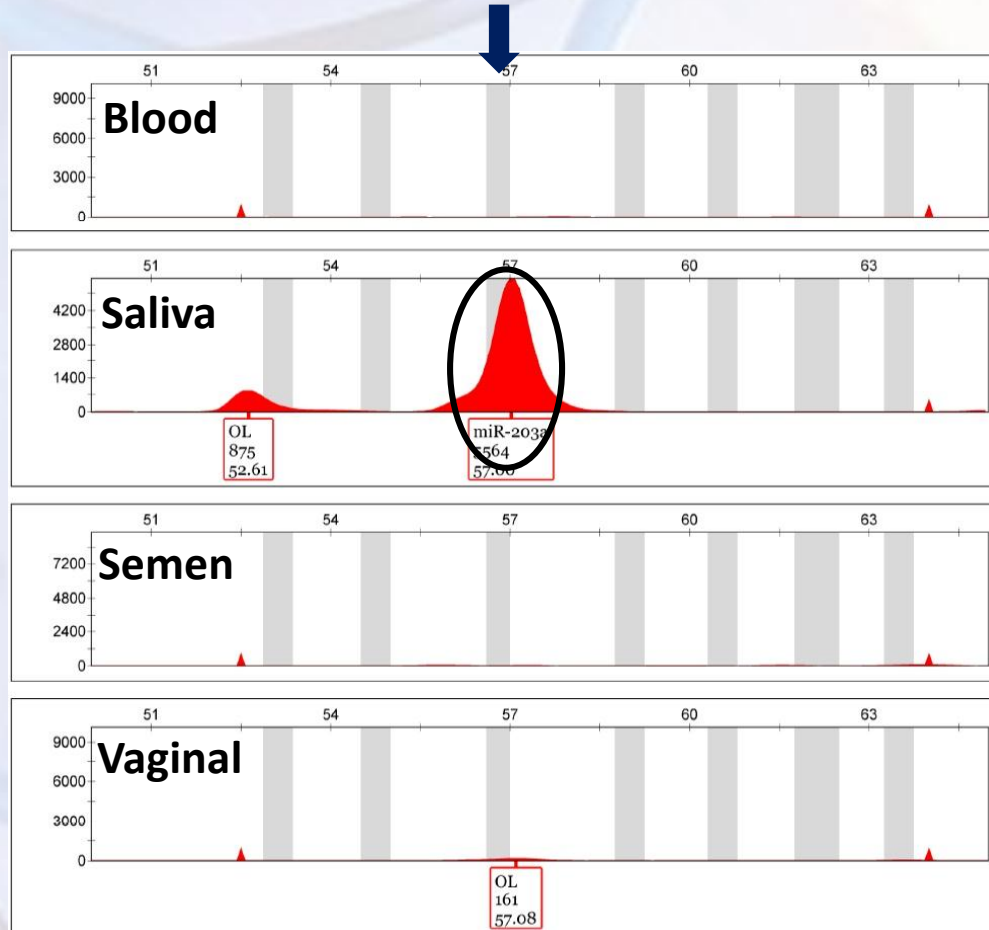
Much lower peaks of
by-products found in all
tissues

miR-203a is mainly detected in saliva

Expressed in epithelial cells

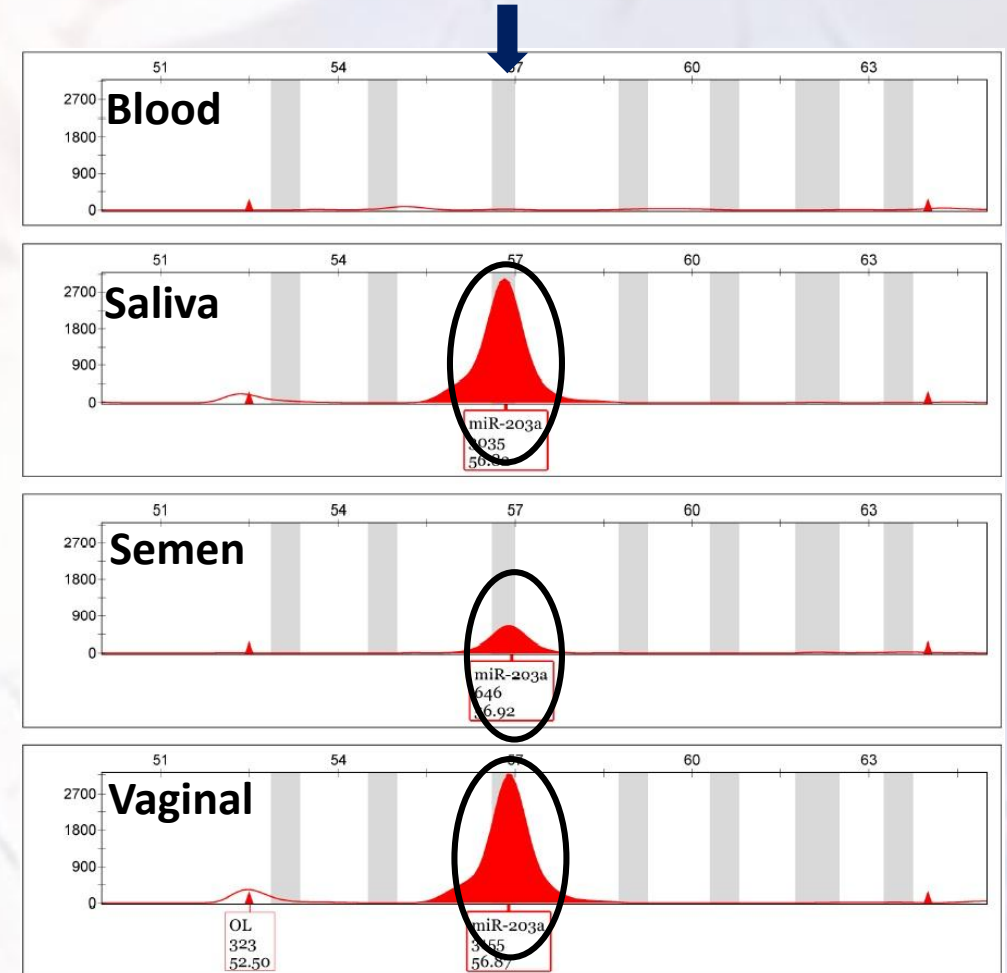
Sample set 1

miR-203a



Sample set 2

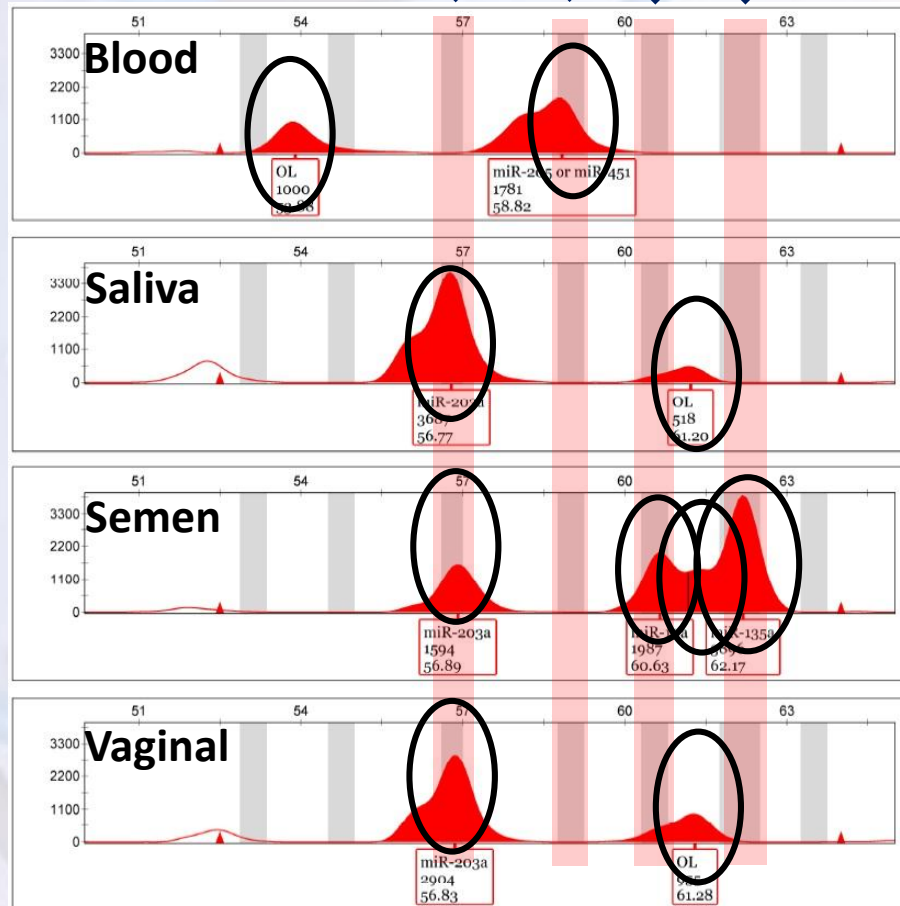
miR-203a



Multiplexing multiple markers yields expected results

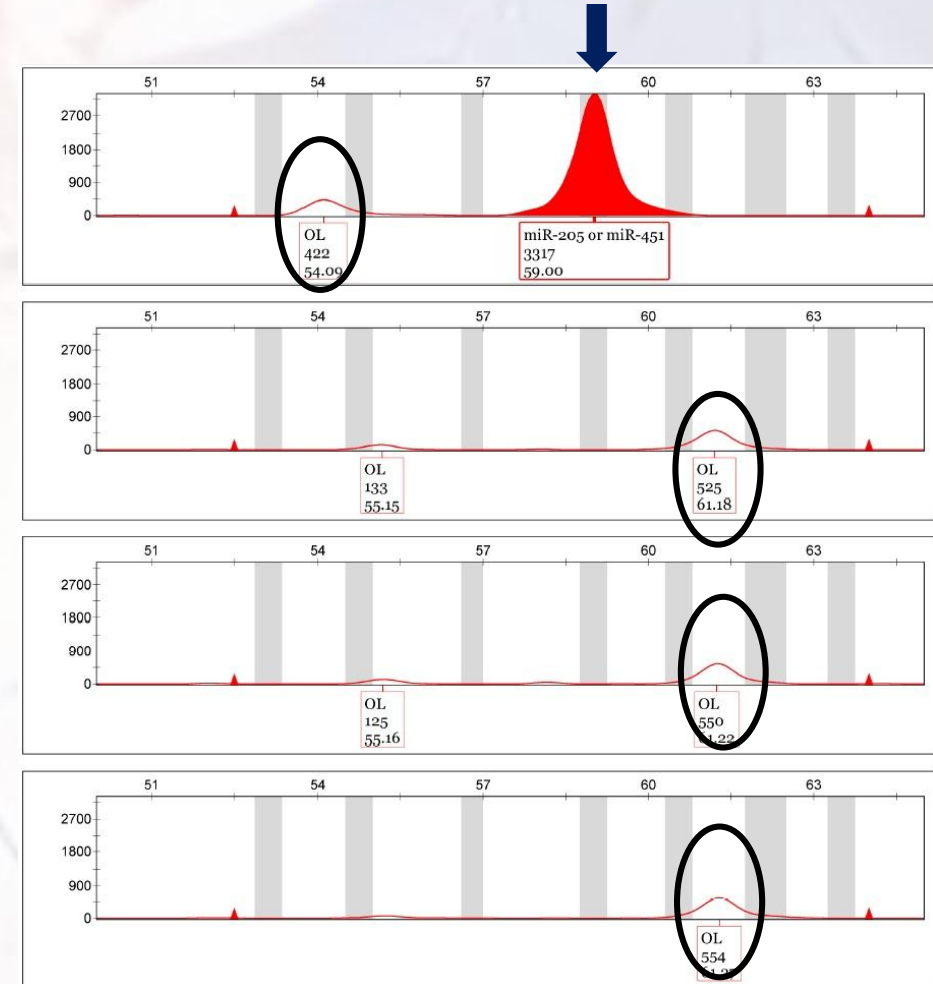
Multiplex

Saliva 203a
Blood 451a
Semen 10a
Semen 135a



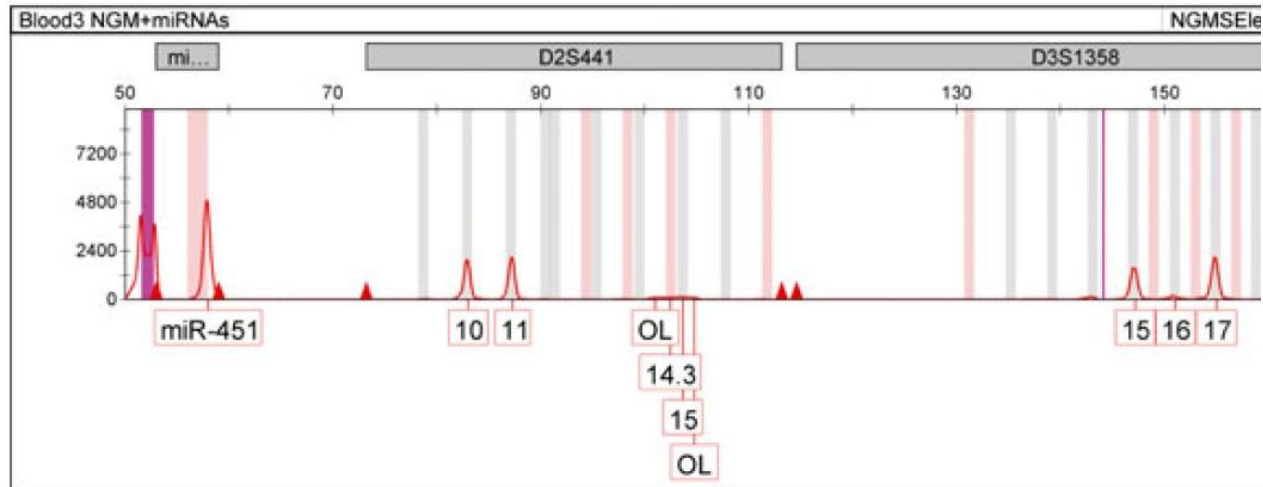
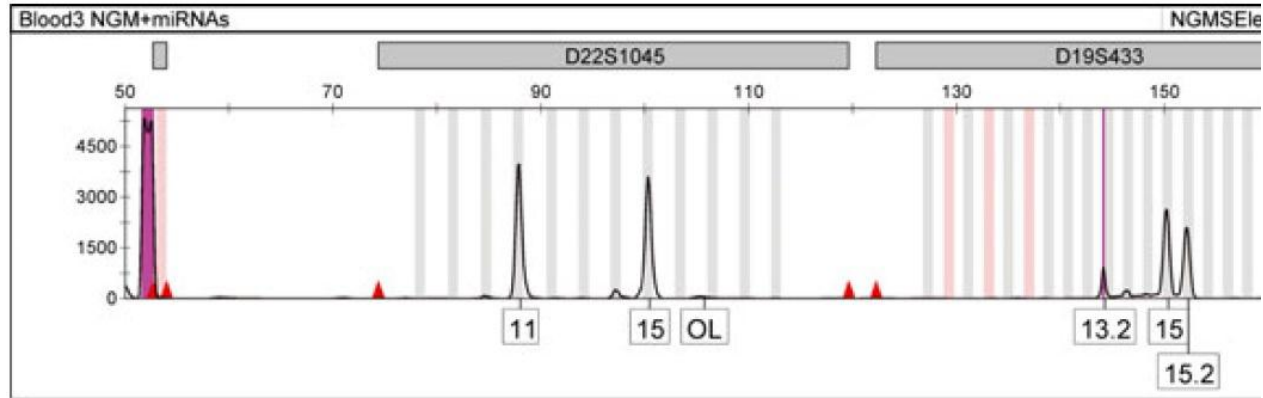
Singleplex

miR-451a

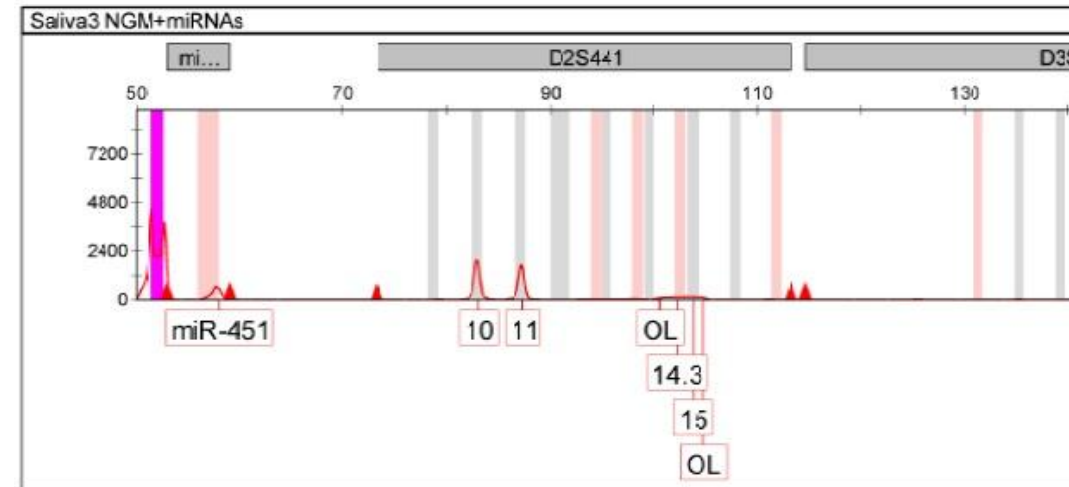
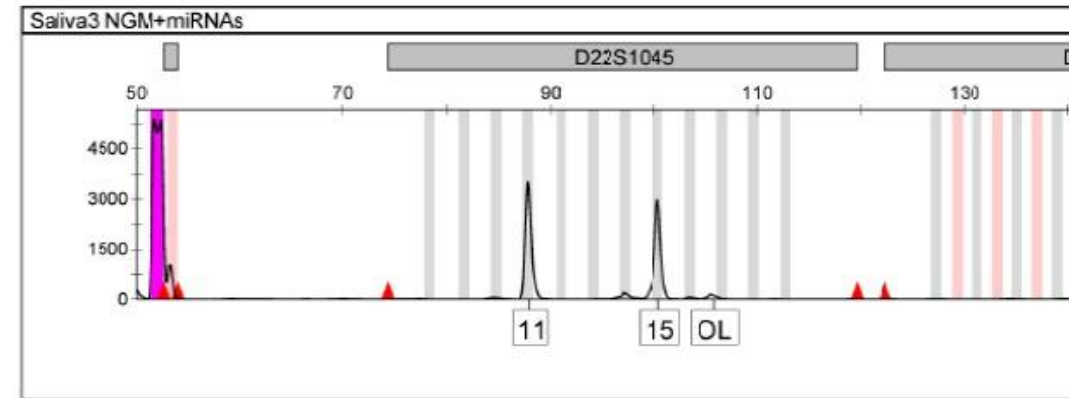


Multiplex with STR markers

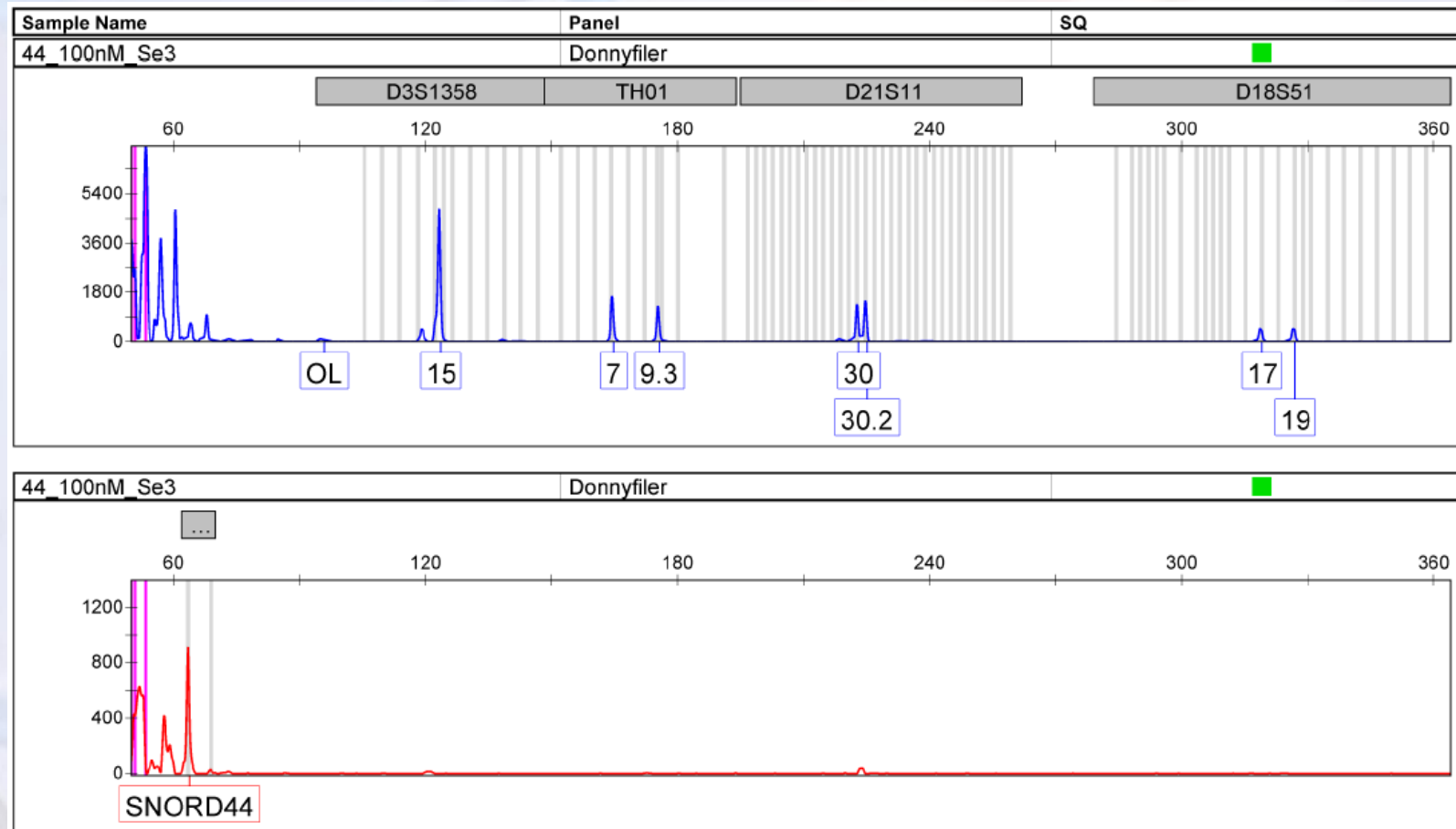
Blood



Saliva



Multiplex with STR markers



Conclusion

- Analysing microRNAs with CE is viable
- Potential for future single confirmatory test
- Combining microRNA analysis with DNA profiling is technically feasible

Future work

- Reduce non-specific amplification
- Physically separate markers
 - Increase product length
- Optimise multiplex reaction
- Combination with DNA profiling

The background features a soft, artistic illustration of DNA double helix structures. One prominent helix is in the upper left, rendered in blue and brown. Another is in the lower left, also in blue and brown. To the right, there's a more complex, multi-colored molecular structure with yellow, orange, and blue components. The overall color palette is light and airy, with pastel blues, yellows, and pinks.

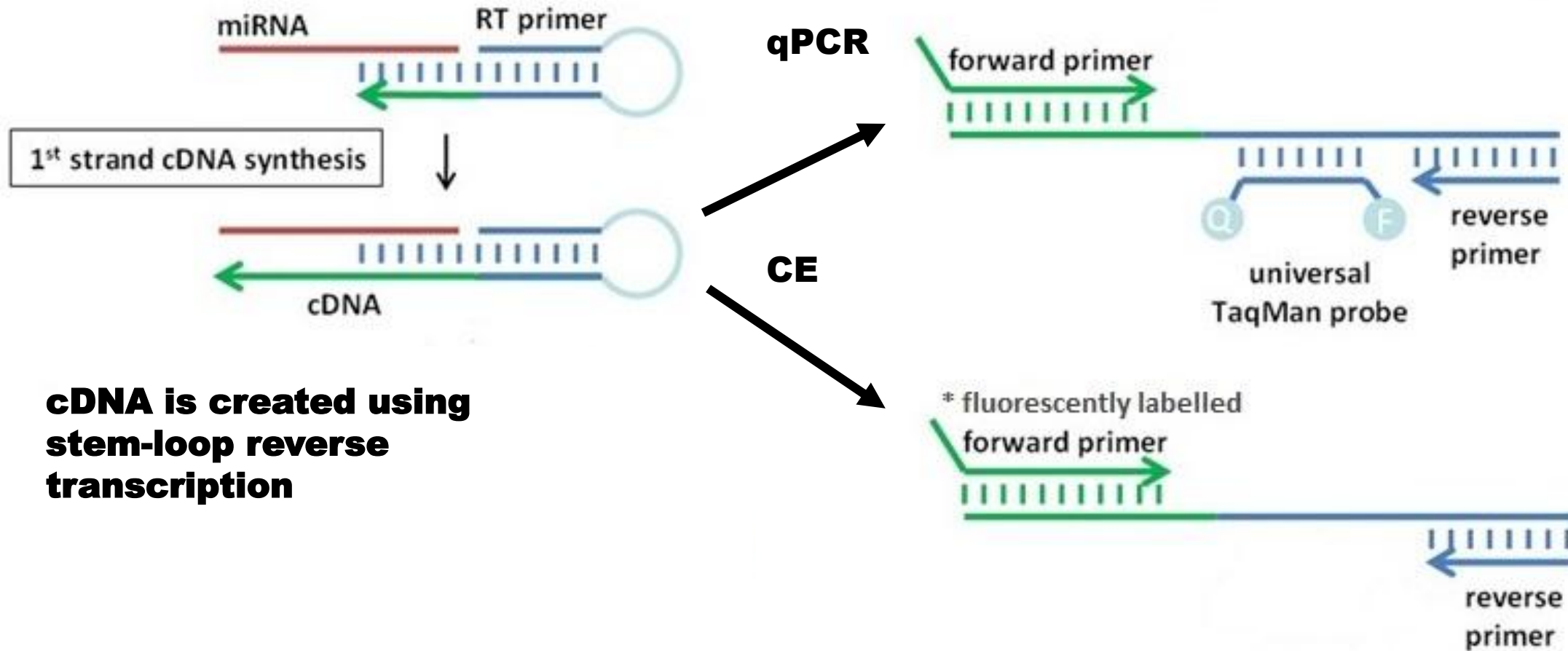
Thank you

d.vandermeer@hud.ac.uk



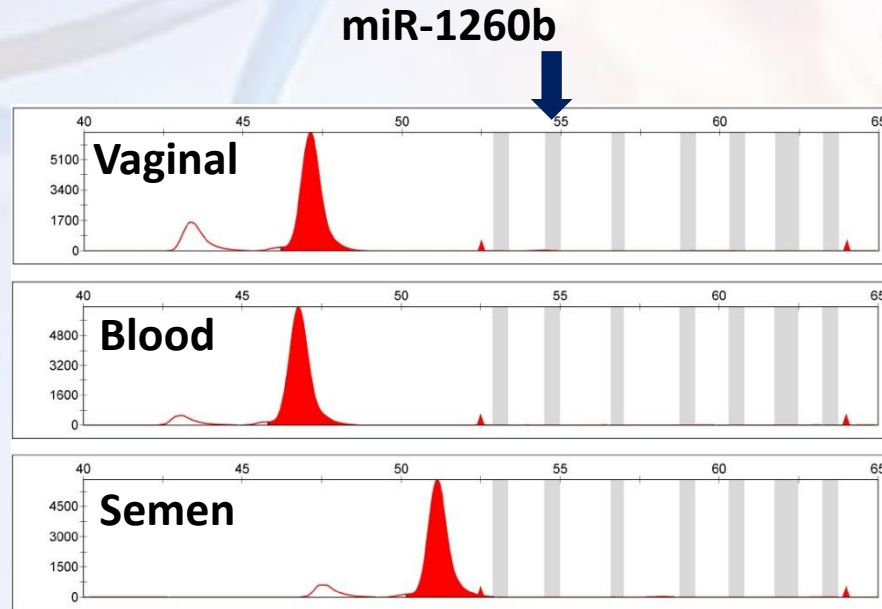
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Our workflow

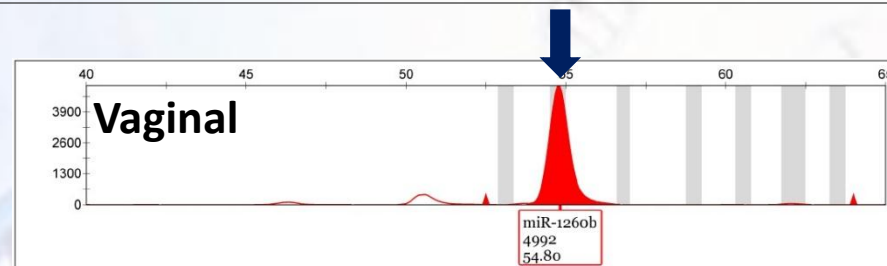


miR-1260b and miR-205 fail due to multiplexing reverse transcription

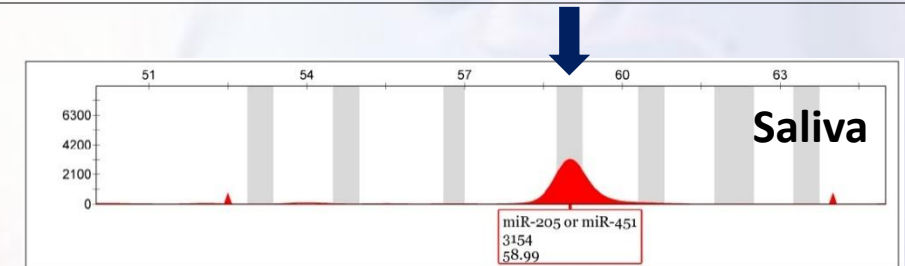
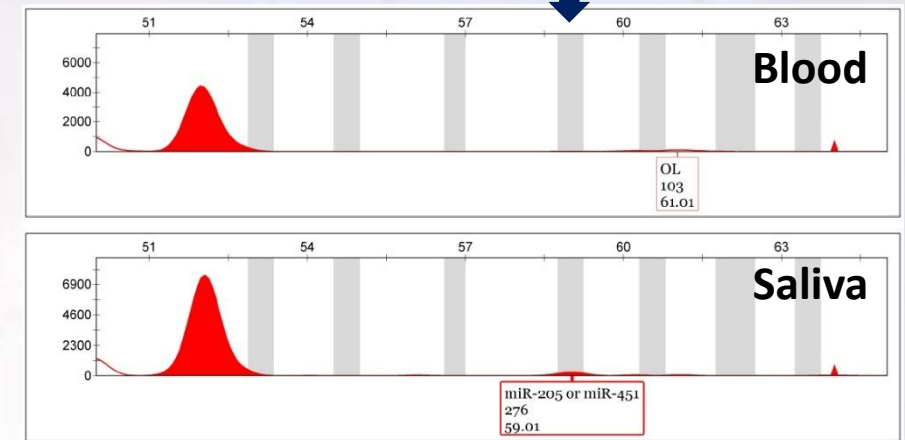
Multiplex RT



Singleplex RT



miR-205



Multiplexing multiple markers yields expected results

